

REMARKS

Claims 1-61 are pending in this application. Claims 1-27, 31-48, 50 and 57-61 are withdrawn from consideration. Claims 51-54 have been canceled without prejudice or disclaimer. Claim 28 is amended to better clarify what Applicants regard as the invention. Support for the amendment to claim 28 can be found throughout the specification, but in particular on page 24, lines 15-19. Accordingly, claims 28-30, 49, 55 and 56 are currently under consideration. No new matter is entered by way of this amendment.

Information Disclosure Statement

The Examiner has noted that the IDS as originally filed and on record, was considered and signed by the previous Examiner in the parent application, but has not been considered and signed by the present Examiner. In order for the references to be listed on the face of the issued patent, Applicants submit herewith an IDS for consideration and signature by the present Examiner.

Rejections under 35 USC 102(b)

The Examiner has maintained the rejection of claims 28-30, claim 49, and claims 55-56 under 35 U.S.C. 102(b) as being unpatentable over Engleman et al. (WO 94/02156). Applicants respectfully traverse the Examiner's rejection and provide further distinctive amendments and arguments herewith, as well as a Declaration under 37 CFR 1.132, in support of the patentability of the present claims. Furthermore, for the convenience of the Examiner, Applicants also provide a pre-print of a reference by Blachère et al. in support of the patentability of the present claims, which is enclosed herewith as Exhibit B.

Applicants' Invention as Currently Claimed

The present invention is directed to methods of assessing cytotoxic T lymphocyte activity comprising providing antigen presenting dendritic cells prepared by contacting apoptotic cells expressing an antigen or apoptotic cell fragments, blebs, or bodies containing the antigen whereby dendritic cells internalize the antigen and present it to a population of T lymphocytes to be assayed for their ability to exhibit killer cell activity and subsequently assaying the cytolytic activity of the T cells. The antigens of the invention may be tumor antigens or viral antigens and the means by which apoptosis is induced may be selected from

a variety of procedures including, but not limited to, ultraviolet light, gamma irradiation, steroids, serum deprivation, cytokines, or drugs which induce apoptosis. Furthermore, the antigen may be produced recombinantly.

More particularly, claim 28 has been amended to recite that the apoptotic cells expressing the antigen of interest are shown to be apoptotic by using particular procedures, such as Annexin V staining, propidium iodide staining, DNA laddering, or staining with dUTP and terminal transferase.

Engleman et al.

As noted previously and maintained in the present Office Action, the Examiner alleges that Engleman et al. teach a method of inducing cytotoxic T lymphocyte activity comprising contacting antigen presenting dendritic cells with a variety of antigen donors including bacterial, parasitic, fungal, viral, and tumor antigens. The antigens may be purified, recombinant, or exist as whole organisms or cells in viable or dead form. The Examiner further alleges that the reference teaches exposing antigen presenting dendritic cells to a population of T lymphocytes to be assayed for their ability to exhibit killer cell activity and assaying the cytotoxic activity of the T lymphocytes exposed to the antigen presenting DCs.

The Examiner further alleges that although the reference does not specifically teach contacting the dendritic cells with “apoptotic cells”, Engleman et al. teach that pulsing DCs includes contact with live or irradiated cells. Furthermore, the Examiner alleges that Applicants teach that one means of inducing apoptosis is by irradiation.

Claim Amendments and Arguments in Support of Patentability

Applicants respectfully traverse the Examiner’s rejection and assert that in order for a rejection under 35 U.S.C. 102(b) to be proper, a single reference must teach each and every element of the invention as claimed. Engleman et al. do not teach the methods of the present invention as currently claimed. There are distinct differences between the teachings of Engleman et al. and the present application.

For example, claim 28, as currently amended recites:

“.....wherein the apoptotic cell may be shown to be apoptotic by a procedure selected from the group consisting of Annexin V staining, propidium iodide staining, DNA laddering, and staining with dUTP and terminal transferase (TUNEL staining),....”

The intent of Engleman et al. was not to induce apoptosis by irradiation of tumor

cells prior to exposure to dendritic cells for cross-presentation to T cells. Further proof that Engelman et al. do not contemplate irradiation for inducing apoptosis is found in the Examples on pages 29- 34 of the reference, in particular, in sections 7 and 7.2, whereby Engelman et al. specifically **do not teach irradiation of the antigens, in particular, keyhole limpet hemocyanin (KLH), sperm whale myoglobin (SWM) and HIV gag peptide antigens, prior to exposure to the dendritic cells for T cell activation.** More importantly, Engleman et al. **do not transfer these antigens to dendritic cells by way of an apoptotic cell.** The antigens of Engelman et al. are presented to the dendritic cell in the absence of an apoptotic cell. Therefore, Engelman et al. **do not teach or suggest inducing or assessing T cell activation by way of an apoptotic cell in the context of a dendritic cell,** as Applicants claim.

As noted in Applicants' response to the previous Office Action, dated October 4, 2004, it is apparent that Engelman et al. did not appreciate the complexity of the methods necessary for optimizing the induction or assessment of cytotoxic T lymphocyte killing activity by delivering antigen to a dendritic cell by way of an apoptotic cell. In fact, it was only through the work of the present inventors that such unexpected findings became apparent. For example, as disclosed in the instant application, dendritic cells are noted in the present application as having the ability to efficiently phagocytose apoptotic cells expressing the desired antigen or apoptotic cell fragments, blebs, or bodies containing antigen, and presenting them in the context of HLA antigens for efficient induction and assessment of T cell responses. Once again, Engelman et al. **did not irradiate antigens nor did Engelman et al. present antigens to dendritic cells by way of an apoptotic cell.** Engelman et al. failed to recognize that antigen may be delivered to a dendritic cell via phagocytosis of an apoptotic cell expressing the antigen for which a T cell response was desired and could be presented in the context of a dendritic cell to T cells for optimal T cell stimulation. One cannot contemplate a solution without having an appreciation for the problem.

Moreover, unlike Engelman et al., **Applicants provide proof that the antigen is being presented to the dendritic cell in the context of an apoptotic cell and that the cell is truly apoptotic by measuring apoptosis as currently claimed.** For example, the Examiner's attention is drawn to page 45, lines 7-12, and to Figure 3a, wherein apoptosis was confirmed using Annexin V binding. Further proof of apoptosis can be found on page 68, lines 1-24, wherein apoptosis was confirmed using the Early Apoptosis Detection Kit (Kayima Biomedical). Cells are stained with Annexin V-FITC and Propidium Iodide,

wherein cells that are apoptotic are Ann V+/PI- as determined by FACScan (Becton-Dickinson) and further by TUNEL staining.

Moreover, while Engleman et al. do not teach the method of irradiation proposed, the irradiation referred to in Engleman et al. was in all likelihood gamma irradiation, which is a procedure standardly used by those skilled in the art to render tumor cells replication incompetent prior to use as a vaccine, or to render the cell preparation free of harmful agents, such as viruses or bacteria. Such cells would not be as efficient as true apoptotic cells in presenting antigen for uptake by dendritic cells, as noted in the instant application. The Examiner's attention is drawn to the present application on page 46, lines 24-33 continuing on to page 47, lines 0-8, wherein it is explained that **apoptotic cells, but not necrotic cells were capable of transferring antigen to the dendritic cells for generation of a potent influenza specific CTL response.**

However, as noted in the present application, and in the Declaration under 37 CFR 1.132 by Dr. Matthew Albert, the use of any irradiation, gamma or ultraviolet, may result in either death of the cell by necrosis or in apoptosis, and the outcome is dependent on the doses used as well as the time of exposure. Applicants clearly point out in the present application, as noted previously and repeated below, the particular methods by which apoptosis is induced, and where irradiation is used, the particular doses and times of exposure.

For example, in the present application, on page 31, lines 19-27, it is noted that:

"Those skilled in the art will recognize that optimal timing for apoptosis will vary depending on the donor cells and the technique employed for inducing apoptosis. Cell death can be assayed by a variety of methods known in the art including, but not limited to, fluorescence staining of early markers for apoptosis, and determination of percent apoptotic cells by standard cell sorting techniques."

Furthermore:

"In one embodiment, donor cells are induced to undergo apoptosis by irradiation with ultraviolet light. Depending on the cell type, typically exposure to UV light (60 mJules/cm²/sec) for 1 to 10 minutes induces apoptosis. This technique can be applied to any cell type, and may be most suitable for a wide range of therapeutic applications. The apoptotic donor cells expressing an antigen of interest on their surface could then be used to prime dendritic cells in vitro or in vivo."

More particularly, the methods of inducing apoptosis by UVB irradiation, as shown by the inventors of the instant application, can be found on page 21, lines 0-3, wherein it

states:

“HeLa cells were labeled with PKH26-GL, followed by irradiation using a 60UVB lamp [Derma Control Inc.], calibrated to provide 240 mJ cm⁻² in 2 minutes, sufficient for the induction of apoptosis.”

Applicants respectfully remind the Examiner that it is a fundamental axiom of the patent law that a reference must enable its alleged teachings in order to serve as a proper reference under 35 U.S.C. 102. Applicants submit that Engleman et al do not enable methods of inducing apoptosis since not only is the type of irradiation not indicated, but there are no specified dosages presented, nor times of exposure. In addition, Engleman et al. **do not teach** methods of inducing or assessing the induction of a T cell response through use of an apoptotic cell for delivery of the antigen to a dendritic cell. Applicants on the other hand teach methods wherein **apoptosis is measured using various procedures, including but not limited to, Annexin V staining, propidium iodide staining, DNA laddering, or staining with dUTP and terminal transferase (TUNEL staining)**. Furthermore, the instant application points out specific and relevant differences in necrotic cells as compared to apoptotic cells as related to their use for either antigen presentation or maturation of the dendritic cell. It is Applicants’ contention that Engleman et al. did not appreciate the difference between use of either an apoptotic cell or a necrotic cell for purposes of inducing or assessing a T cell response. No one could appreciate these differences in outcome of responses until the time of the present invention.

Applicants provide herewith as Exhibit B, a pre-print of a paper by Blachere et al., wherein the authors demonstrate that apoptosis is a live/active cell process. Moreover, this paper demonstrates that proteolysis is necessary to allow for cross-presentation of antigen by apoptotic cells by way of dendritic cells to T cells. (See Figure 2 of Blachere et al.) Applicants also demonstrate that apoptosis inhibitors, such as Z-VAD, blocked T cell activation even in the presence of UV irradiation (see the present application on page 46, lines 4-22 and Figure 3c).

Based on the foregoing, and in summary, Applicants contend that Engleman et al. do not contemplate nor appreciate the need for antigen uptake and transfer to the dendritic cell by way of an apoptotic cell, which is then presented to the T cell in the context of the MHC. Applicants further assert that the Engleman et al publication **does not teach or suggest** the preparation and use of antigen containing apoptotic cells for induction or assessment of T cell activity. Nor do Engleman et al. teach how to prepare apoptotic cells containing the antigen

for delivery to the dendritic cell. In fact, the only antigens taught by Engleman et al are KLH, SWM and HIV antigens, which are not presented in the context of an apoptotic cell. Furthermore, Engleman et al. do not teach that the cells are apoptotic by using the various procedures known to measure apoptosis, including Annexin V, propidium iodide, DNA laddering, or staining with dUTP and terminal transferase (TUNEL staining) as a means of determining whether irradiation, if used, induces apoptosis, as presently claimed by the inventors of the present application. In all likelihood, if Engleman et al. used irradiation to treat tumor cells prior to use as a vaccine, then they most likely used enough gamma irradiation to kill the cells, or to sterilize the preparation of cells, which was not the intention of the present application. In light of the present claim amendments and without the proper teaching of the type of irradiation, as well as the dose or length of time of exposure of tumor cells in Engleman et al., Applicants maintain that Engleman et al. do not teach or suggest the methods of the present invention.

Applicants further assert that the rejection under 35 U.S.C. § 102(b) is improper in that the Engleman et al reference is a non-enabling reference. As stated in In re Donohue, 766 F.2d 531, 533, 226 USPQ 619, 621 (Fed. Cir. 1985):

It is well settled that prior art under 35 U.S.C. § 102(b) must sufficiently describe the claimed invention to have placed the public in possession of it. Accordingly, even if the claimed invention is disclosed in a printed publication, that disclosure will not suffice as prior art if it was not enabling. It is not, however, necessary that an invention disclosed in a publication shall have actually been made in order to satisfy the enablement requirement.

Applicants further assert that the use of apoptotic cells as an entity capable of transferring antigen to dendritic cells for cross-presentation to T cells was unknown prior to Applicants' own work. Engleman et al. do not teach or suggest that the tumor cells noted on page 19 of their patent application were apoptotic as shown through use of one of the procedures for measuring apoptosis described above and presently claimed in the instant application.

It is Applicants' contention that Examiner has tried to reconstruct Applicants' invention using hindsight reconstruction, which is impermissible.

In light of the foregoing claim amendments and arguments, Applicants respectfully request withdrawal of the rejection.

Fees

A check in the amount of \$905. is enclosed to cover the small entity fees for the Request for Continued Examination (RCE) and the petition for a three month extension of time. No other fees are believed to be due for the present response. However, should this be in error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment, or to credit any overpayments.

Conclusion

Applicants believe that the foregoing amendments to the claims place the application in condition for allowance. Withdrawal of the rejections and objections is respectfully requested. If a discussion with the undersigned will be of assistance in resolving any remaining issues, the Examiner is invited to telephone the undersigned at (201) 487-5800, ext. 118, to effect a resolution.

Respectfully submitted,



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Attachments: Declaration under 37 CFR 1.132;
Exhibit A: CV of Dr. Matthew Albert;
Exhibit B: Pre-print of a paper by Blachere et al., PLoS Biology, June 2005,
Vol. 3;
Information Disclosure Statement